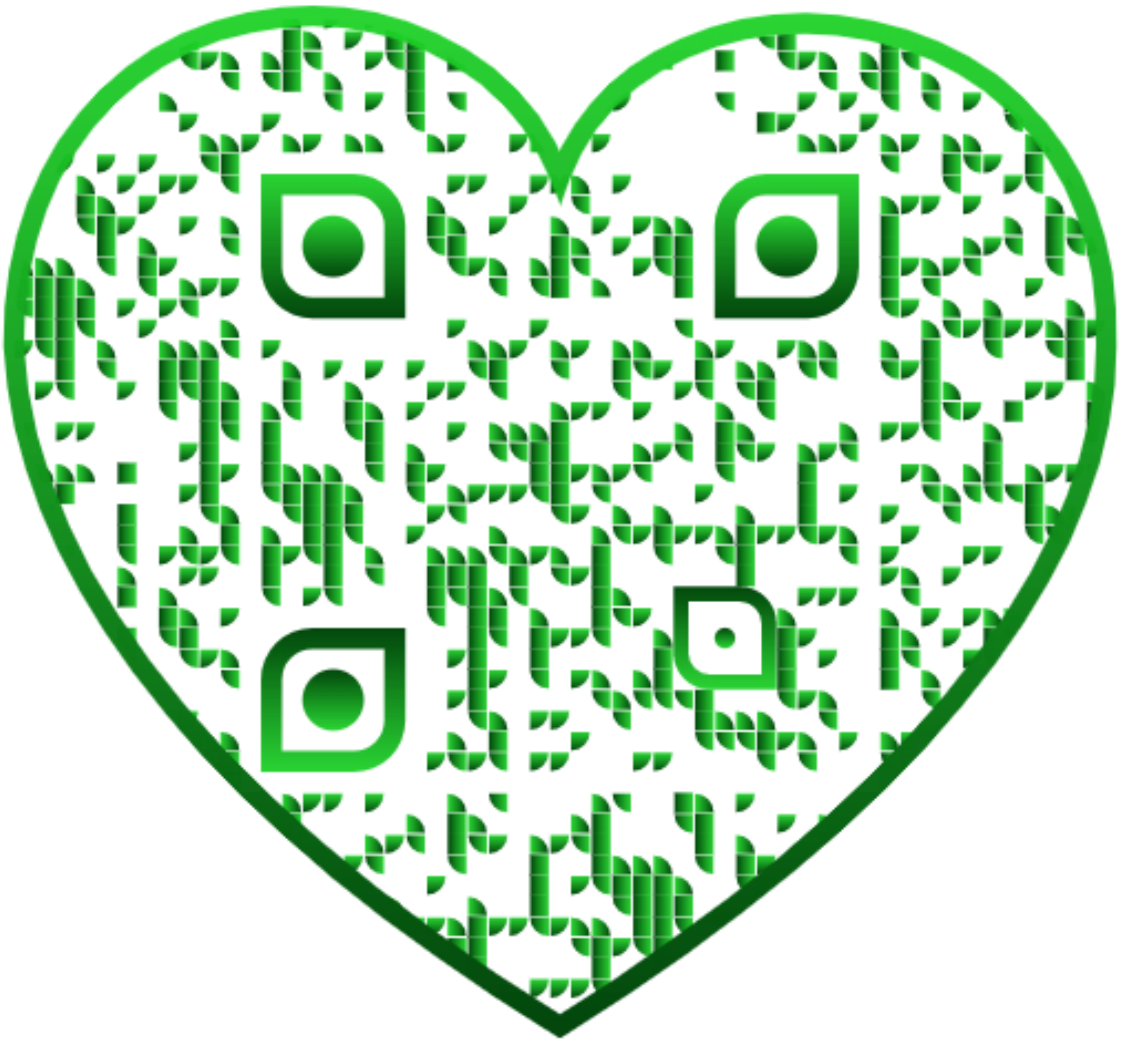
Practical Medical Microbiology

Part 2

Dr/ Abeer Nasher



Dr/ Abeer AM Nasher

Simplified Definition of the Immunological Laboratory

- ***Immunological Tests:*** **these tests** employ an antigen to detect presence of antibodies to a pathogen, or an antibody to detect the presence of an antigen, of the pathogen in the specimens of the victim.

Immunology and serology laboratories focus on the following:

- **Identifying antibodies.** These are proteins made by a type of white blood cell in response to a foreign substance (antigen) in the body.
- **Investigating problems with the immune system.** These include when the body's immune system attacks its own tissues (autoimmune diseases) and when a body's immune system is underactive (immunodeficiency disorders).
- **Determining organ, tissue, and fluid compatibility for transplantation**.

Diagnostic immunological methods rely on:

a. Direct detection of microbial antigens in specimens with florescent-labeled antibodies.

b. They are used in routine diagnostic microbiology for the rapid diagnosis of serious infection such as → ***Bacterial Meningitis***

c. When a pathogen difficult or impossible to culture →***e.g. Chlamydia trachomatis***

***Define of* some terms that found in practical immunological lab.**

1. ***Serology*:** it is called on the study of antigen-antibody reactions in vitro (study of sera).

   ↳ ***(The clear fluid that separates when blood clots).***

2. ***In vitro means***: the study of Ag-Ab reactions **outside** of the body.
3. ***In vivo means:*** the study of development of antibody against specific foreign antigen within body.
4. Antibody: a group of plasma proteins (immunoglobulin) that are found as results of exposure to antigen. Types of antibodies are IgG, IgM, IgA, IgE and IgD.
5. Antigen: they are toxin produced by foreign bodies that are mostly proteins, polysaccharides and glycolipids, which stimulate the immunity system to produce the antibodies.

✹ ***Briefly notes for safety in this lab.***

There are some procedures must be follow that called are (SOPs).

↳ Standard operating procedures mean all safety rules and safe work practices in lab. These procedures should be established to:

1. Meet specific operating needs.
2. Reduce the risks associated with lab. hazards.

- **As a condition of entry to a lab. All individuals must complete**:

1. A lab. safety induction such as care to prevent the infection of lab. workers with pathogens.
2. Receive specific training (effective training) in local safety rules and lab. procedures at relating to their work.
3. Individuals are required to comply (obey) with lab. safety rules and procedures at all the time whilst in lab. by put planning.

❖ **The safety rules for Lab. depend on two things:**

1. *The kind of laboratory it is.*
2. *The level of understanding of the people working in it.*

- ***General procedures are used in all laboratories:***

1. Personal protective equipment (PPE): wear lab. coat, gloves, closed shoes and eye protection (chemical splash goggles).
2. Never mouth pipette.
3. Cover any cuts or burns.
4. Do not eat or drink in lab.
5. In case of any accident, reports to instructor.
6. A void hand to face operation.
7. Wash hands before you leave.

- ***Material that cause the infections such as:***

1. Live cultures.
2. Inculcated culture media.
3. Hypodermic needles.

- ***Lab. hazards and accidents:***

a. Infections → ***the ways***→ inhalation, ingestion and inoculation.
b. Burns → ***the ways***→ chemical and Bunsen burner.
c. Hormel effect of toxic chemical.
d. Electric shock.

- ***Hazardous Microorganism (M.O):***

❖ ***Classified into four groups:***

1. Low risk→ fungi (***Pencillium***)
2. Moderate risk → bacteria (***Staphylococci*)**.
3. High risk→ drugs or vaccine → (***Salmonella***)
4. High risk→ no vaccine → viruses (***HIV***)

Immune Serological Testing

- Numerous types of serologic tests
- differ in their speed and sensitivity.
- Some are strictly **qualitative**, others are **quantitative**.
- <u>These Tests include:</u>

1. Precipitation
2. Agglutination
3. Neutralization
4. Complement fixation
5. Immunofluorescence
6. Radioimmunoassay (RIA)
7. Enzyme-Linked Immuno sorbent Assay (ELISA)
8. Western Blotting

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Immunochromatography Technique(lab.1)

Introduction

Immunochromatography assay (ICA), namely lateral flow test, is a simple device intended to detect the presence or absence of the target analyte (antigen or antibody). The concept (principle)of immune-chromatography is a combination of chromatography (separation of components of a sample based on differences in their movement through a sorbent) and immunochemical reactions. The most widespread immunochromatographic system is the test strip (Figure 1).

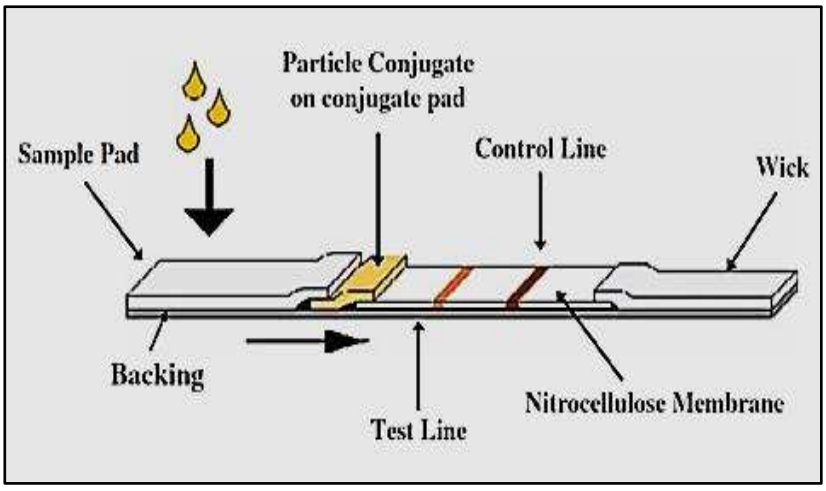


Figure 1. Typical layout of a lateral flow test strip.

Developing an ICA

Strips or Cassette used for ICA contain four main components:

1. Sample Application Pad(in the sample well)

It is made of cellulose and/or glass fiber and sample is applied on this pad to start the assay. **Its function is to transport the sample to other components**. Sample pad should be capable of transportation of the sample in a *smooth, continuous and homogenous manner*. This pretreatment may include:

- Separation of sample components,
- Removal of interferences,

- Adjustment of the pH, etc. Analyte sample should be added to the sample application pad to start the test.

2. Conjugate Pad.

It is the place where labeled bio recognition molecules (labeled antibodies, usually Nano colloid gold particle) are dispensed. Material of conjugate pad should immediately release labeled conjugate upon contact with moving liquid sample. Labeled conjugate should stay stable over entire life span of the lateral flow strip. ***Any variations in dispensing***, ***drying or release of conjugate can change the results of assay significantly***. Poor preparation of labeled conjugate can adversely affect sensitivity of the assay. *Glass fiber*, *cellulose*, polyesters and some other materials are used to make conjugate pad.

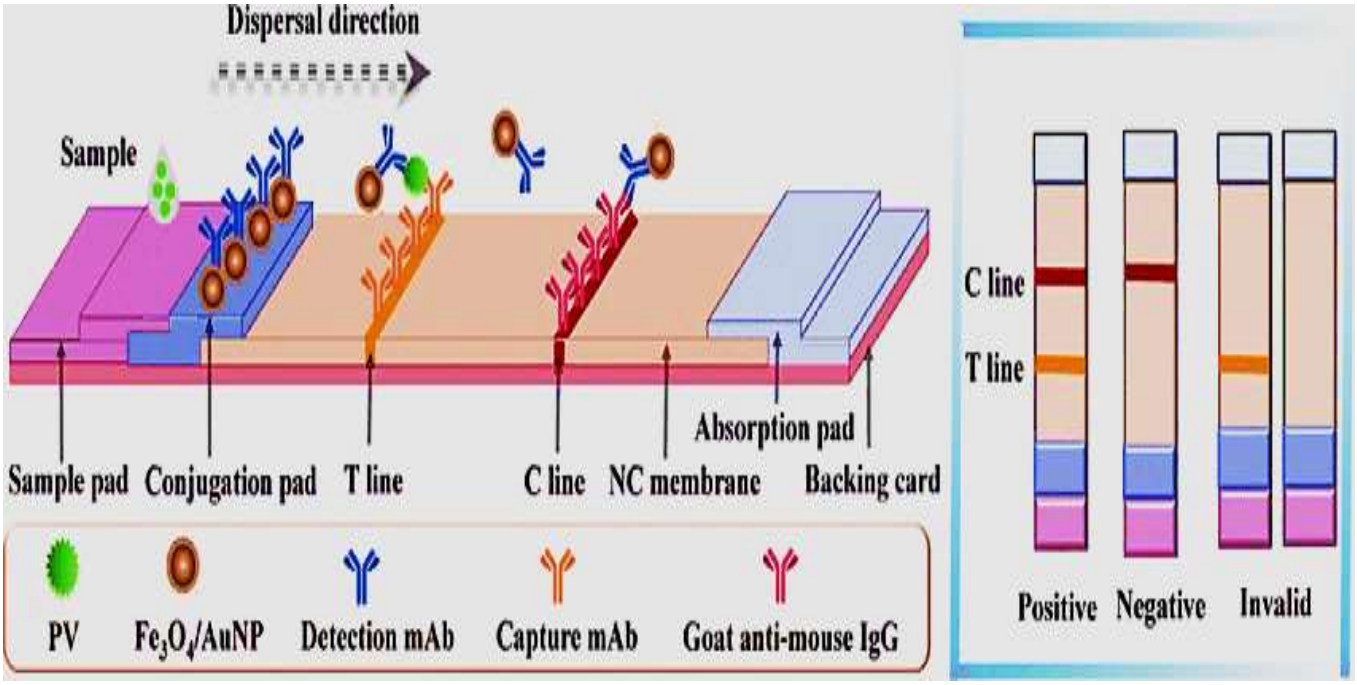
3. Substrate(Nitrocellulose) Membrane.

It is highly critical in determining sensitivity of ICA. Test and control lines are drawn over this piece of membrane. So an ideal membrane should provide support and good binding to capture probes (antibodies, etc.). Nonspecific adsorption over test and control lines may affect results of assay significantly, thus a good membrane will be characterized by lesser non-specific adsorption in the regions of test and control lines. Proper dispensing of bioreagents, drying and blocking play a role in improving sensitivity of the assay.

4. Adsorbent Pad

It works as sink at the end of the strip. It also helps in maintaining flow rate of the liquid over the membrane and stops back flow of the sample. Adsorbent capacity to hold liquid can play an important role in results of assay.

All these components are fixed or mounted over a backing card. Materials for backing card are highly flexible because they have nothing to do with ICA except providing a platform for proper assembling of all the components. Thus, backing card serves as a support and it makes easy to handle the strip.



Major steps in ICA are:

1. Preparation of labeled antibody and capture antibody against target analyte;
2. Immobilizing the labeled antibody onto conjugate pad, and the capture antibody onto the strip membrane to form the Test/Control line.
3. Assembling of all components onto a backing card after dispensing of reagents at their proper pads.
4. Add samples and buffer onto sample pad.
5. Wait the sample flow through the test and control line for 5-10 min.
6. Read the result when the color reveal.

ICA Format

Lateral flow assay basically combines a number of variants such as formats, bio recognition molecules, labels, detection systems and applications. There are types of ICAs based on detection format, in this lab. You take one type of it:

1. Sandwich Assay

In this assay format, label (Enzymes or nanoparticles or fluorescence dyes) coated antibody is immobilized at conjugate pad. This is a temporary adsorption which can be flushed away by flow of any buffer solution. A capture antibody against target analyte is immobilized over test line. A secondary antibody against labeled antibody is immobilized at control zone (Figure 2).

To start a test,

Sample containing the analyte is applied to the sample application pad and it subsequently migrates to the other parts of strip.

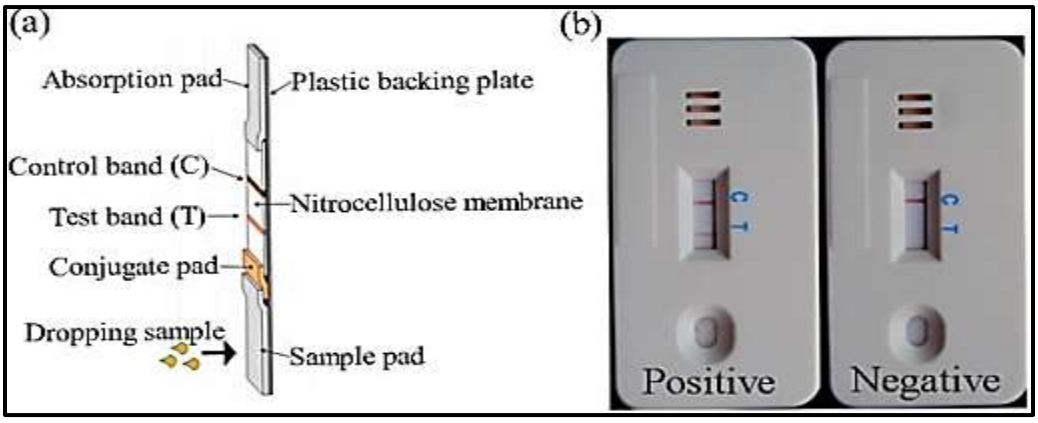
At conjugate pad, target analyte is captured by the immobilized labeled antibody and results in the formation of analyte-labeled antibody complex.

This complex now reaches to nitrocellulose membrane and moves under capillary action. At test line, analyte-labeled antibody complex is captured by another antibody which is primary to the analyte. Analyte becomes sandwiched between labeled and primary antibodies forming labeled antibody-analyte-primary antibody complex.

Excess labeled antibody will be captured at the control zone by secondary antibody. Buffer or excess solution goes to absorption pad.

Intensity of color at test line corresponds to the amount of target analyte and is measured with an optical strip reader or visually inspected.

Appearance of color at control line ensures that a strip is functioning properly.



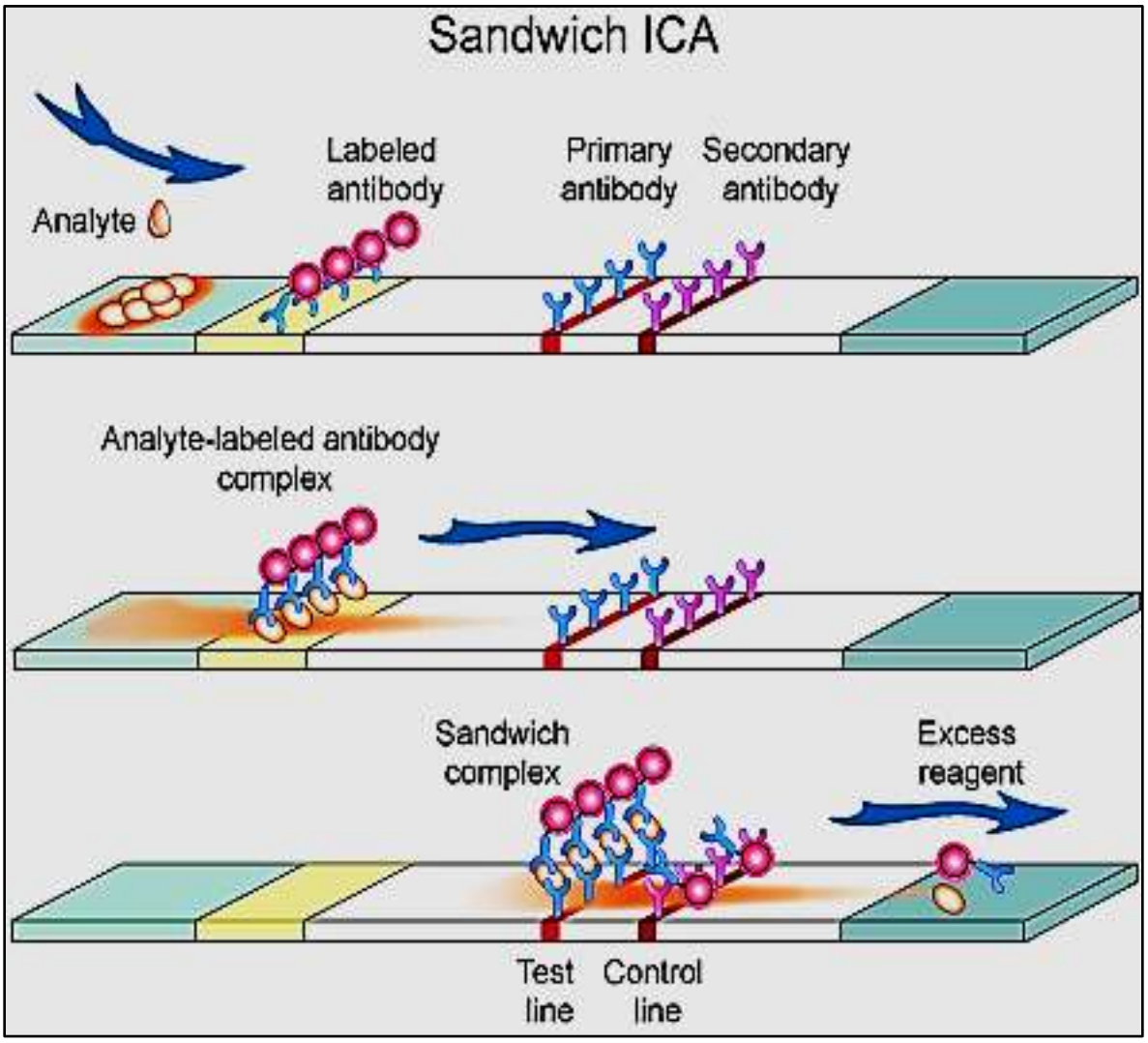


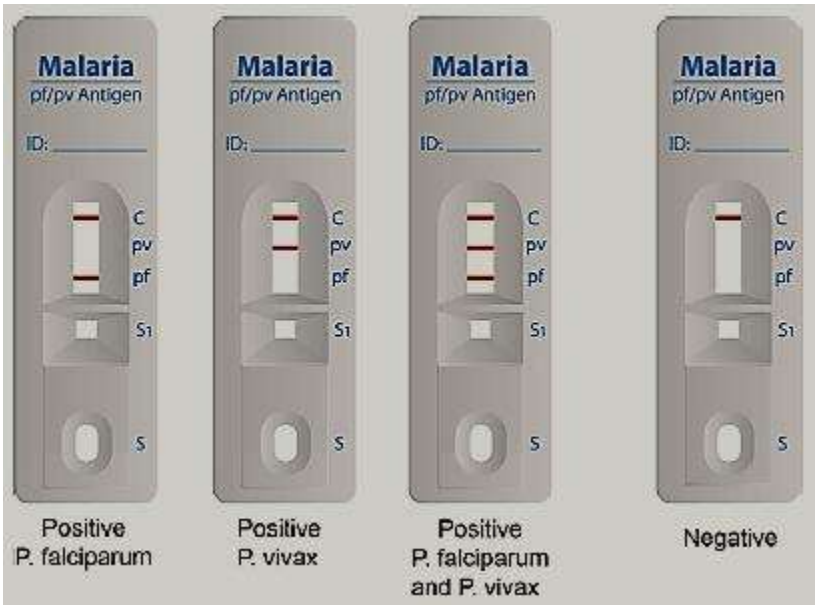
Figure 2. Schematic diagram of Sandwich ICA.

Tests are scanned :

1. HIV 1/2 Ab Cassette.
2. HCV Ab Cassette.
3. HBsAg Cassette.

Specimens →Whole blood/ serum/ plasma

4. **Urine** pregnancy test (**Strip**) and (**Cassette**) .
5. H. pylori Ab (**Cassette**). Specimens →Whole blood/ serum/ plasma
6. Malaria Pf/Pv Ab Cassette. Specimens →Whole blood/ serum/ plasma



Interpretation of the test: (Strips or Cassette)

The test kit has two line control and test line on the surface of the cassette or strip. Both of these lines are not visible before applying any samples. The control line always appears if the test procedure is performed.

- ***Negative result:*** the presence of only one (red or purple) band within the result window indicates a **negative** result that means *antigens* ***or*** antibodies are **absent** in the specimen.
- ***Positive result:*** the presence of two color bands ("T" & "C") within the result window, no matter which band appears first, indicates a **positive** result that means antigens or antibodies are **present** in the specimen.

- ***Invalid result:*** If the red or purple color band is not visible within window after performing the test, or one line appear on test band but absent the control band indicates the test result is **invalid** that means:

  1. The directions may not have been followed correctly.
  2. Insufficient (not enough) specimen volume.
  3. The test kit is expired.

→ **In this Case**, it is recommended that the specimen be re-tested by using a new device

REQUEST FOR: IMMUNOLOGICAL ASSAY

Name Of technique	Observation	Result
1.		
2.		

Interpretation:

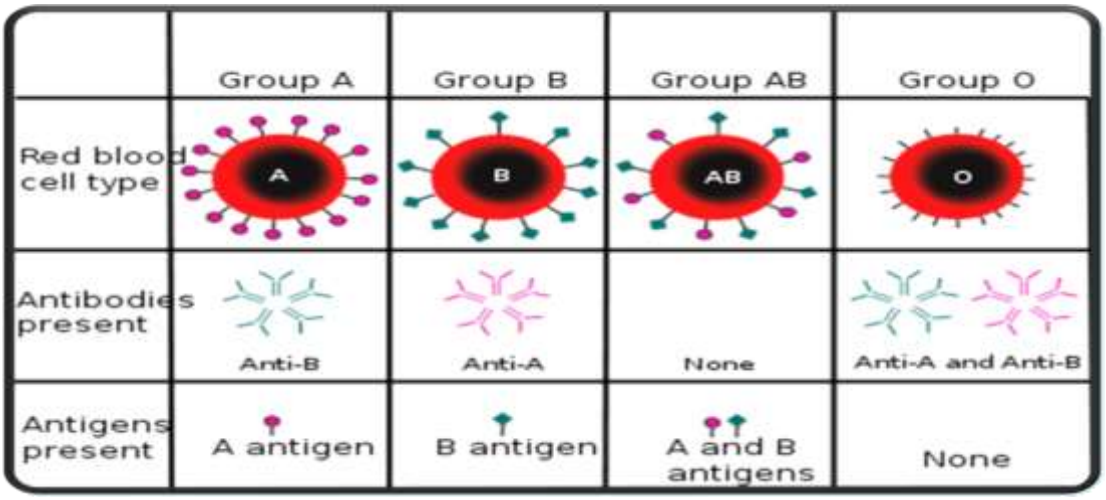
1. Do you think the cassette number (2) is positive? Interpretation?
   _____
   _____
2. Study the cassette number (3) and determine the result of it?
   _____
   _____
3. Read the result in your cassette and interpret it?
   _____
   _____
   _____
4. The result in the cassette of number (3) is positive :
   a. True. Why? _____
   b. False. Why? _____
5. Name the target analyte in strip number (1):_____

The Blood Group Systems(lab.2)

What are the different blood groups?

- The differences in human blood are due to the presence or absence of certain protein molecules called antigens and antibodies.
- The antigens are located on the surface of the RBCs and the antibodies are in the blood plasma. which will attack certain antigens if they are present.
- The blood group you belong to depends on what you have inherited from your parents.
- There are more than 20 genetically determined blood group systems known today
- The ABO and Rhesus (Rh) systems are the most important ones used for blood transfusions.
- Not all blood groups are compatible with each other. Mixing incompatible blood groups leads to blood clumping or agglutination, which is dangerous for individuals.
- According to the ABO blood typing system there are four different kinds of blood types: A, B, AB or O .
- Group O is the most common (40% of population)
- There are various types of red blood cell antigens. By the time a person is six months old, he/she naturally will have developed antibodies against the antigens his/her red blood cells lack.

Blood Types

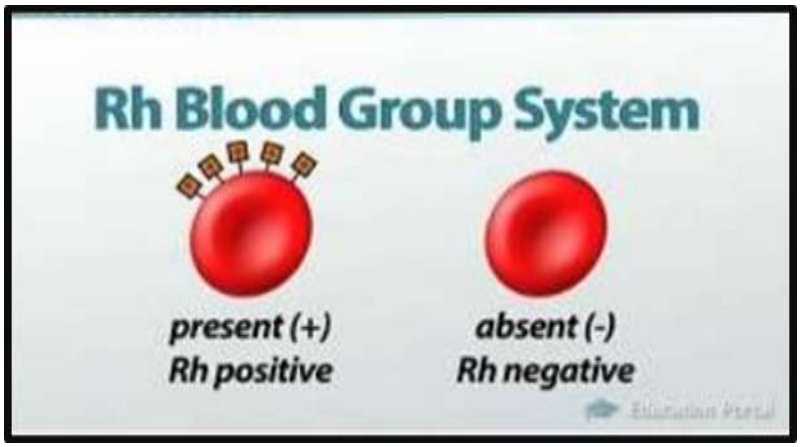


1. O can be given to anyone but can only receive O.
2. AB can receive any type but can only be given to AB.
3. Also, every person's blood is either Rh-positive or Rh-negative

❖ The Rhesus (Rh) Blood Group system

- ♦ This is next importance blood group system, whereas name Rhesus comes from the Rhesus monkeys in which the protein was first discovered.
- ♦ The Rh blood group is determined by its genotypes that classified to, D which is a strong and d is a weak. There are DD, Dd, and dd which can be found.
- ♦ Rh refers to the presence or absence of the D antigen on the red blood cell.
- ♦ D is dominant while d is recessive, so that genotypes DD, Dd are Rh+ve (positive) while genotype dd is Rh - ve (negative).
- ♦ The presence or absence of D antigen determines if the person is Rh+ or Rh -

- Rhesus factor D (antigens), the most important, is found in the blood of 85% of people and the remaining 15% are Rhesus negative. So people can be classified according to both systems, for example AB positive or O negative.
- Unlike the ABO system, individuals who lack the D antigen do not naturally produce anti-D.
- Production of antibody(anti-D) to D requires exposure to the antigen.
- The D antigen is very immunogenic, i.e. individuals exposed to it will very likely make an antibody to it.
- These antibodies are formed only in the plasma of Rh-ve persons in certain cases:
    1. If they are transfused with Rh+ve blood.
    2. If they be given the protein breakdown products of Rh+ve red blood cells, because the Rhesus factor is the foreign antigen in Rh-ve persons.
- For this reason all individuals are typed for D, if negative must receive Rh (D) negative blood.



The following table shows the compatibility of blood types between blood donors and recipients.

Blood types that match	
A person who has	***Can receive***
A- blood	A-, O- blood
A+ blood	A-, A+, O-, O+ blood
B- blood	B-, O- blood
B+ blood	B-, B+, O-, O+ blood
AB- blood	AB-, O- blood
AB+ blood	AB-, AB+, A-, A+, B-, B+, O-, O+ blood
O- blood	O- blood
O+ blood	O-, O+ blood

The transfusion reaction:

- ***Blood received in*** *a transfusion must have* ***the same antigens****(**compatible blood**).*

- *If you get the transfusion that has different antigens (**incompatible blood**), **the antibodies in your plasma will destroy the donor blood cells**. This is called a **transfusion reaction**, and it occurs immediately when incompatible blood is transfused.*
- *The transfusion reaction can be **mild** or **cause a serious illness** and **even death**.*
- ***Type O-negative blood does not have any antigens**. It is called the **"universal donor"***
- ***Type AB-positive blood** is called the **"universal recipient"** type because **a person who has it can receive blood of any type**. Although the two types may be **used to classify blood in an emergency**. Blood type tests are always done to prevent transfusion reactions.*

How to Find Your Blood Group?

❖ *Principle* :

*The principle of ABO grouping **is based on a specific agglutination** reaction between **antigens on red cells (blood specimen) and IgM antibodies in the typing serum (anti-serum)***

Slide Method for ABO grouping

— ***Steps of Procedure:***

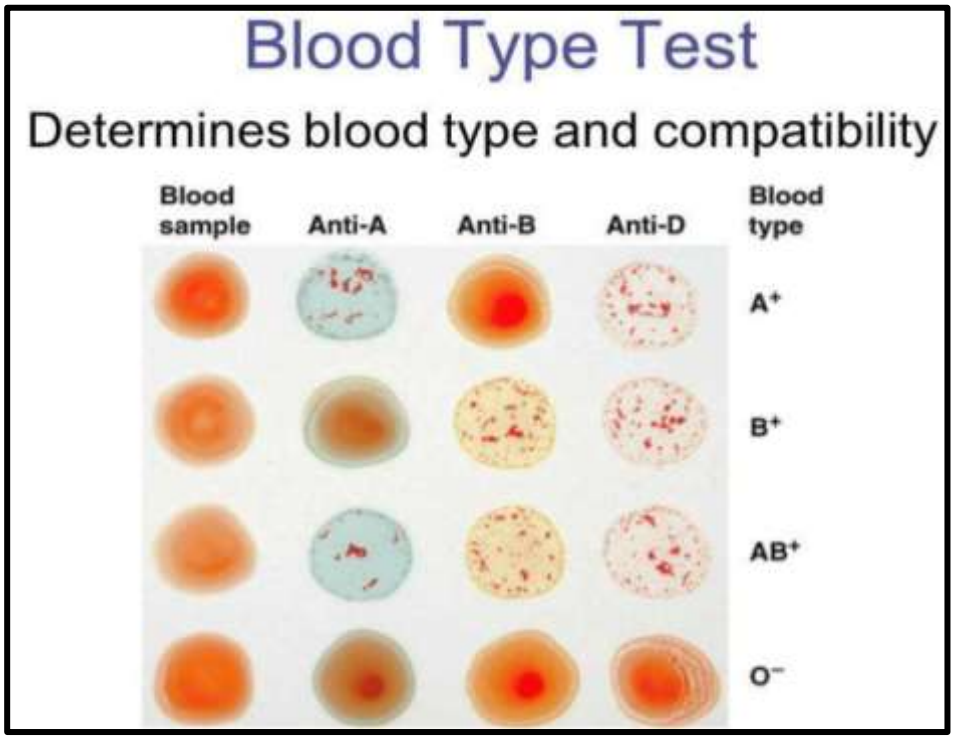
1. *Put "anti-A", anti-B and anti-D on the on the section of slide labeled by anti- A , B and D.*
2. *Add 1 drop of test red cells (blood sample) to each drop of typing antisera.*
3. *Carefully mix each solution with a separate side of the applicator stick*
4. *Slope the slide slowly, back and forth, for two minute at room temp (20-24 ^{o}C) s. Make sure that with each tilt the antibody/RBC mixture falls complete to the end of the slide before tilting in the opposite direction.*
5. *Record results as POS, if agglutination occurs or NEG, if there was no agglutination.*

Important definitions:

- *A false negative is a reaction which gives a negative result when in fact the substance (antigen) being tested was present.*
- *A false positive is a reaction which gives a positive result when in fact the substance (antigen) is not present yet agglutination occurs.*
- *It is important to read the results immediately as false positives can occur when the mixture begins to dry on the slide.*

Interpretation:

- *Agglutination (clumping) of the red blood cells is positive and indicates the presence of the antigen to which the antibody is directed.*
- *No agglutination is negative and indicates absence of the antigen to which the antibody is directed. (Determine the name of antigen)*



ABO and Rh-factor Slide Agglutination Test

Report and Exercise

a. **Record your reactions for samples 1 and 2 in the spaces below. Write "*positive*" if agglutination was observed, write "*negative*" if no agglutination occurred. Use the table on the previous page to interpret your results.**

Name	ID #	Anti-A	Anti-B	Anti-D	Observation
•	1				
•	2				

- **Interpretation(Why)?**

Determination of C-reactive protein(CRP), Antistrptolysin-O(ASO)and Rheumatoid Factor(RF) in non-diluted Serum (lab.3)

SLIDE AGGLUTINATION TECHNIQUE

- **Clinical Significance of (CRP):**
  - This protein is ***an acute phase protein present in normal serum***, which increases significantly after most forms of tissue necrosis, inflammation, bacterial and viral infections and malignant neoplasia.
  - It is one of the **humoral factors** (tissue defenses of an innate immunity) that ***plays important role in resolution of inflammatory process*** by ***the rapidity of it's response to injury.***
  - CRP is produced in the liver and its level is measured by testing the blood.
  - Determination of CRP is of great value in diagnosis, treatment and monitoring of inflammatory conditions.
  - The CRP test is sensitive indicator for inflammatory processes, e.g. for rheumatic fever and for the acute phase of rheumatic arthritic.
  - ***CRP concentration ‹ 6 mg/l in the sera of healthy adults***, in a number of disease states these values often exceeded within 4 to8 hours after an acute event and reach levels up to 500 mg/l.

- **Clinical Significance of (ASO):**
  - Streptolysin O is a ***toxic immunogenic exoenzyme*** produced by ***B-hemolytic Streptococci of groups A, C and G.***
  - Antistrptolysin-O ***antibodies*** are ***produced*** due to ***the presence of streptolysin O antigen*** liberated by the previous bacteria.
  - *An ASO concentration less than 200 IU/ml*,
  - ***Measuring the ASO antibodies*** are useful for the diagnostic of rheumatoid fever, acute glomerulonephritis and streptococci infection.
  - ***Rheumatic fever*** is ***an inflammatory disease affecting connective tissue*** from several parts of human body as ***skin, heart, joints***, etc….and acute glomerulonephritis is a renal infection that affects mainly to renal glommerulus.
  - Information on the extent and degree of infection can be obtained from the measurement of serum ASO levels.

- **Clinical Significance of (RF):**
  - Rheumatoid factors are a heterogeneous group of high molecular weight IgM molecules (***auto antibodies***) directed **against** the ***antigenic sites on the Fc portion*** by ***body's own immunoglobulin***(the patient'IgG).
  - RF is ***abnormal protein*** occurs ***in the serum*** of many ***patients suffering from rheumatoid arthritis.***
  - Rheumatoid arthritis is a ***systemic disease*** characteristic by swelling, pain and chronic inflammation of joints lead destruction of joints tissues and resultant joints deformity.
  - ***RF test allows clinicals to distinguish between R.A and rheumatic fever in which RF is almost always absent.***
  - Between ***60-80%*** of patients with ***active rheumatoid arthritis*** (RA), ***possess*** this ***abnormal protein*** in ***their blood and joint fluid.***

Intended Use:

CRP: We use diagnostic reagent for the ***qualitative and semi-quantitative determination*** of human CRP of patient by latex agglutination (coated with specific lgG anti-human CRP antibodies)

ASO: We use diagnostic reagent for the ***qualitative and semi-quantitative determination*** of (ASO) antibodies in human serum by latex agglutination

RF: Used for the qualitative screening and Semi-quantitative test determination of Rheumatoid factors in serum as an aid in the diagnosis of rheumatoid arthritis.

Principle of the Test:

- ***CRP:*** The assay is performed by **suspension latex particles coated with specific goat lgG anti- human CRP** against **unknown serum**.

Latex particles (anti- human CRP antibodies) + unknown serum (specimen)

↓

agglutination (antigen-antibody reaction).

Reagents:

1. ***Latex reagent:*** particles coated with specific goat lgG anti- human CRP antibodies
2. ***Control positive:*** human serum with a CRP concentration >20 mg/l.
3. ***Control negative:*** animal serum with maximum concentration of human CRP of 1 mg/l.

- ***ASO:*** The assay is performed by suspension latex particles coated with pure natured human straptolysin-O (toxin) against unknown serum.

Latex particles (stabilized* straptolysin-*O) + unknown serum (specimen)

↓

agglutination (antigen-antibody reaction).

Reagents

1. ***Latex reagent:*** particles coated with straptolysin-O.
2. ***Control positive:*** human serum with a ASO* concentration>200 IU/ml.
3. ***Control negative:*** containing less than 200 IU /ml of ASO*. Human serum.

- ***RF:*** The assay is performed by testing a suspension of latex particles coated with human gamma globulin against unknown serum.

Latex particles (human immunoglobulin G) + unknown serum (specimen)

↓

agglutination (antigen-antibody reaction).

Reagents:

1. ***Suspension of latex:*** particles coated with human gamma globulin.
2. ***Control positive:*** human serum containing RF concentration greater than > 8 IU/ml (1 drop→50ul).
3. ***Control negative:*** containing RF concentration less than < 8 IU/ml.

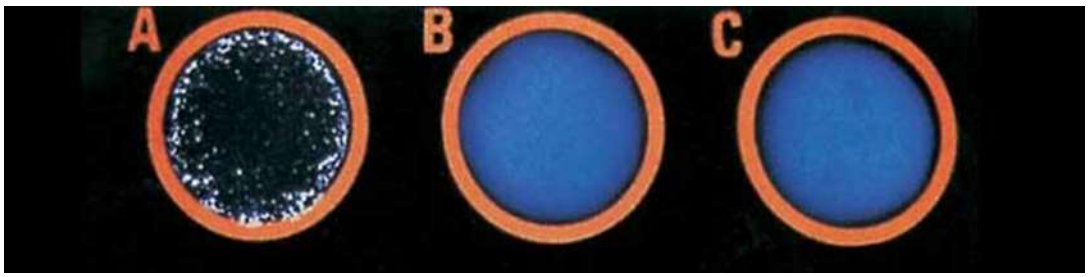
1-Procedure Method 1 (Qualitative technique):

1) Bring the reagents to room temperature. The sensitivity of the test may be reduced at low temperatures.
2) Place 50 ul of the specimen and one of each positive and negative controls into separate circles on the slide test.

3) Swirl the ***CRP, ASO and RF latex reagent*** gently before using and add one drop (50uL) next to the specimens to be tested.
4) Mix the drops with stirrers, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5) Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. Read the presence or absence of visible agglutination in this period of time. False positive results could appear if the test is read later than two minutes. (i.e. unspecific agglutination result).
6) Observe immediately under a suitable light source for any degree of agglutination.
7) The reaction of the test serum is compared to the positive and negative control sera.

Place successively on the slide:

	Positive control	**Sample**	**Negative control**
Latex reagent R1	**50 ul drop**	**50 ul drop**	**50 ul drop**



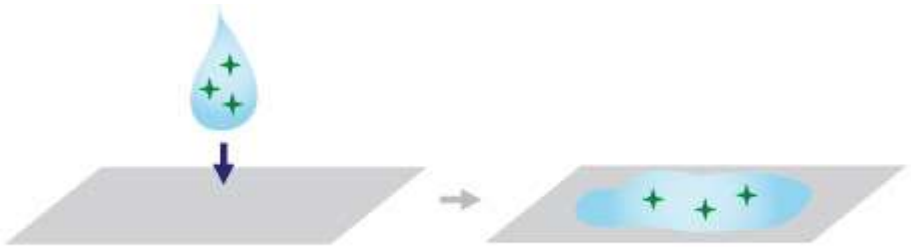
C+ve **specimen test** **C-ve**

Reading and interpretation:

- Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.
- *Nonreactive:* Smooth (uniform milky) suspension with no visible agglutination, as shown by negative control. Lake of agglutination indicates ***a CRP concentration lower than 6 mg/l*** in specimen, also ***an ASO concentration less than 200 IU/ml***, and ***a RF concentration less than 8 IU/ml.***
- *Reactive:* Any degree of agglutination visible macroscopically (Clearly visible aggregates). The presence of agglutination indicates ***a CRP concentration equal or greater than 6 mg/l***, also ***an ASO concentration above 200 IU/ml***., and ***a RF concentration above 8 IU/ml***.
- A weakly reaction serum produces a very fine granulation or a partial clumping.

Slide Latex Agglutination Technique: How it works

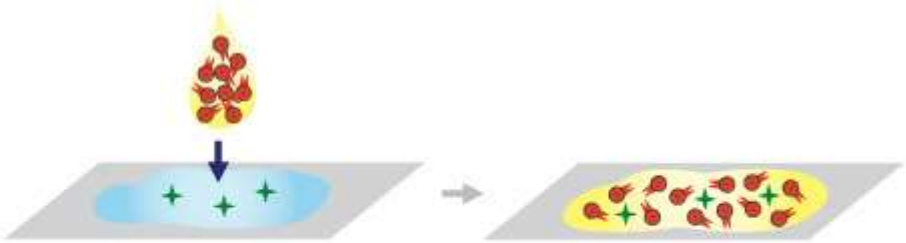
Step 1: Add specimen



A drop of specimen is placed on a card.

Analyte is floating freely in solution.

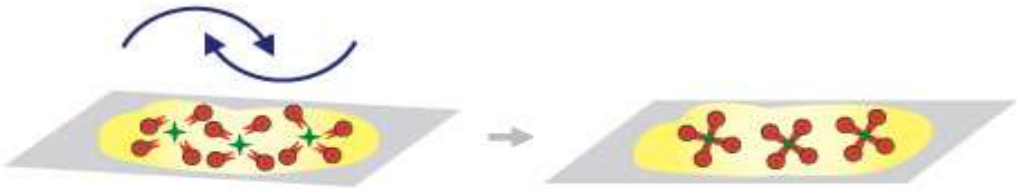
Step 2: Add agglutination solution



A solution which contains an analyte-specific detector molecule attached to a particle is added to the specimen on the card.

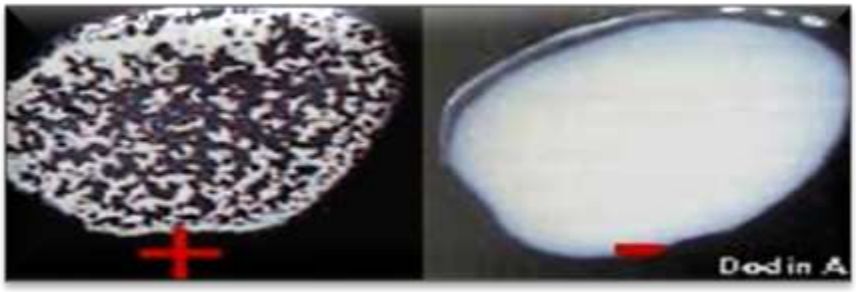
Analyte and detector molecules initially float free of one another causing the color and texture of the drop on the card to be uniform throughout.

Step 3: Agitate (Shake) sample, let settle, and observe for agglutination



The mixture is gently agitated for a number of minutes which causes the analyte and detector molecules to interact.

The interaction of analyte and detector molecules forms detectable aggregates. If analyte were not present in the specimen, aggregates would not form and the color and texture of the mixture would remain uniform throughout.



Request for: immunological assay

Name Of Test	*Observation*	*Result*
1.		
2.		

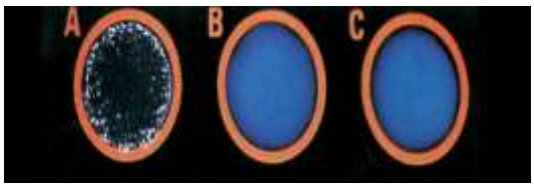
1. **<u>Interpretation→ your observation→(WHY this would happen?):</u>**

---

2. **Name this reagent? What it's useful?**

---

3. **It is abnormal protein** occurs **in the serum** of many **patients suffering from rheumatoid arthritis**.
   a. Name this test_____
   b. Record its level in this specimen_____
   c. Name the target analyte_____



Enzyme Linked Immune-Sorbent Assay**(ELISA. lab.4)**

General information:

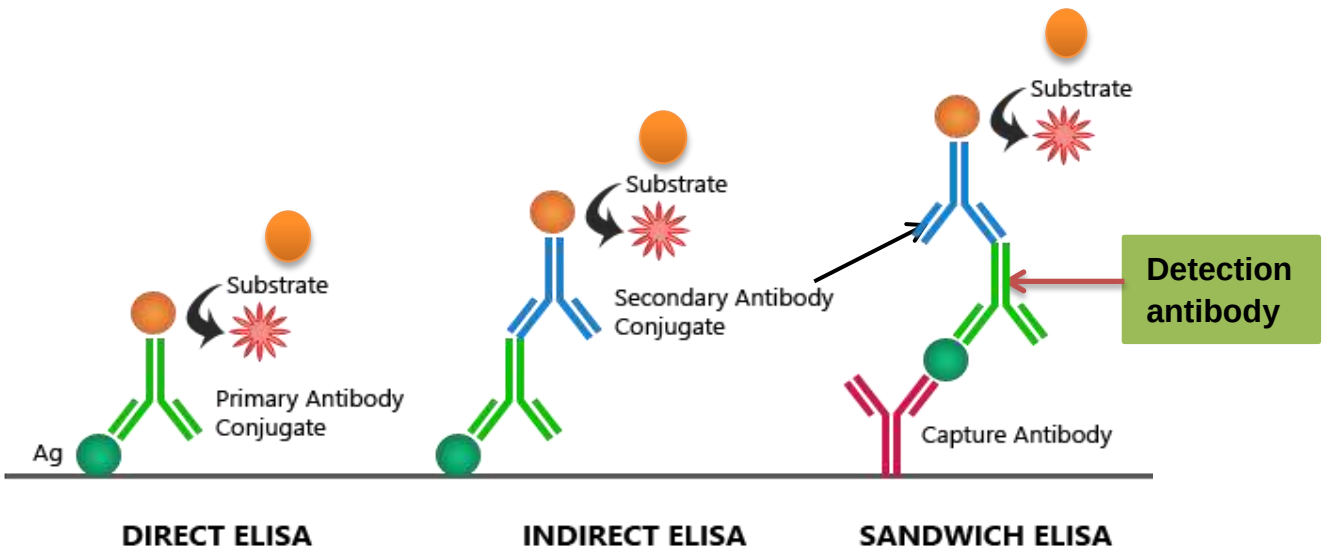
1. The ELISA has been used as a diagnostic tool in medicine laboratories.
2. The ELISA is a rapid test used for detecting and quantifying antibodies or antigens against viruses, bacteria and other materials such as hormones and drugs.
3. ELISA is quick and simple to carry out, and since it is designed to rapidly handle a large numbers of specimens in parallel.
4. ELISA results ***qualitative***(determine antigen or antibody is present or absent.) and ***quantitative*** (determine the quantity of factors, titer and the highest dilution of specimen which gives a positive reaction in the test).
5. **Five** kinds of ELISA are here illustrated as you may concern: *Direct ELISA, Indirect ELISA Sandwich ELISA, Competitive ELISA and ELISpot.*

Basic principle of ELISA Procedure:

There are multi-step of process:

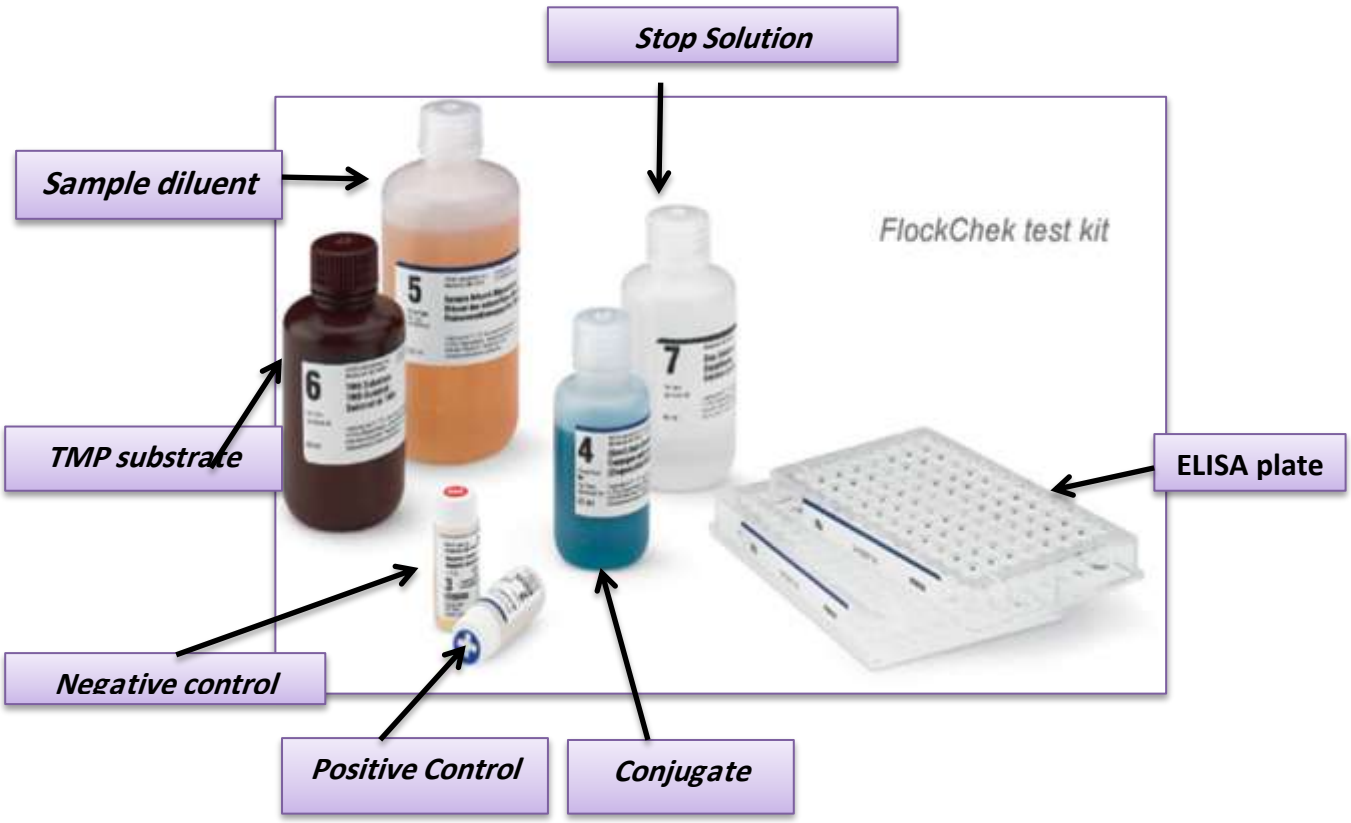
A Coating step, where the first layer, either An antibody or antigen ("immuno") is adsorbed ("sorbent") onto the polystyrene 96 wells plate. Follow this, ***blocking step***, block all unbound sites by factors found in specimen to prevent false positive. Then ***Detection Step***, is used of multiple layers of antibodies to modify signal. ***Results step*** is appeared by the enzymatic reaction will produce a colored species. Color develops in the presence of bound enzyme and the optical density is read with an ELISA plate reader.

In this lab. You know three types of this technique and you know some information about ELISA components.



Format OF ELISA

ELISA Kit:



ELISA Components continued

Coated Plates

The 96-well plates are made of polystyrene and coated with either inactivated antigen or antibody. This coating is the binding site for the antibodies or antigens in the sample. unbound antibodies or antigens in the sample are washed away after incubation.

Sample Diluent

Most assays require a specific dilution of the sample. Samples are added to the sample diluent and mixed prior to putting them onto the coated plates.

Controls

The positive control is a solution that contains antibody or antigen. The negative control is a solution without antibody or antigen. The controls help

- ***to normalize or standardize*** each plate.
- used to ***validate*** the assay and
- to ***calculate*** sample results.

In most tests, the controls are pre-diluted and ready to use. Be sure to follow the instructions in the package insert.

Conjugate

ELISA conjugates are enzyme-labeled antibodies or antigens that react specifically to plate-bound sample analytes. Unbound conjugate is washed away after incubation and before the addition of substrate. The optical density of the colorimetric substrate is directly proportional to the quantity of bound enzyme present.

Substrate

For peroxidase conjugates, the substrate is a mixture of hydrogen peroxide and a chromogen that reacts with the enzyme portion of the conjugate to produce color.

Wash Concentrate

The wash concentrate is a buffered solution containing detergent used to wash away unbound materials from the plates.

Stop Solution

The stop solution stops the enzyme-substrate reaction and, thereby, the color development.

ELISA Equipment

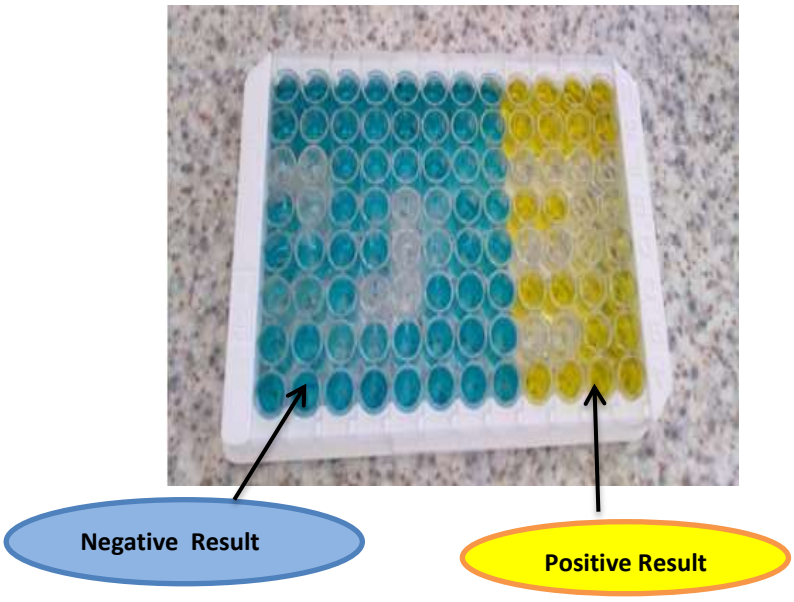
Equipment for ELISA testing is widely available. Readers, washers and pipettes are available as manual or automated systems.

Close Plate Readers

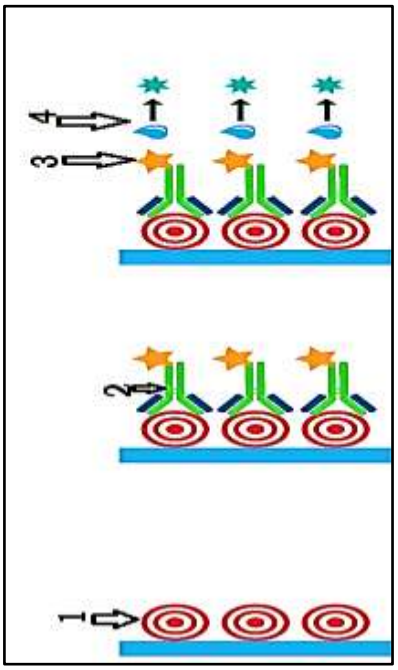
-

Pipettes

Automatic microplate washers washer



REQUEST FOR : IMMUNOLOGICAL ASSAY



These pictures exhibits one of a biochemical technique used mainly in immunology to detect the presence of antibody or antigen in patient sample.

Record your answer according to these questions.

1. ***Determine the format of these ELISA technique.***
   - Direct technique ( )
   - Sandwich technique ( )
   - Indirect technique ( )

_____

2. ***Determine the number of these terms, according to your first answer.***
   - Capture antibody ( )
   - Substrate ( )
   - Adsorbed antigen ( )
   - Enzyme conjugate ( )
   - Primary antibody ( )
   - Detection Antibody( )
3. ***Name this device and what its purpose?***

_____

4. ***Name this equipment and write its function?***

_____

5. ***Study the following bottles and names them?***

_____
_____
_____
_____
_____

Dr/ Abeer Nasher

Fungus Diagnostic Tests(lab.5)

Introduction

What is mycology ?

Mycology is the study of fungi. i.e. study of molds (filamentous organisms) and yeasts (single cells which produce progeny by budding , usually. The disease caused by fungi is called Mycoses.
Fungal toxins : Mycotoxicology

What are fungi ?

Eukaryotic that is, they have a membrane - enclosed nucleus , and other membrane bounded organelles, spore – bearing, heterotrophic that produce extracellular enzymes and absorb their nutrition.
Both sexual (meiotic) and asexual (mitotic) spores may be produced , depending on the species and conditions. Fungi do not photosynthesis. Fungi are a diverse group of saprophytic and parasitic organisms that varies in complexity and size.
Cell wall and membrane components : Fungal cell wall are composed largely of Chitin, a polymer of N acetyl-glucosamine , rather than peptidoglycan . The fungal membrane contains ergo-sterol , rather than the cholesterol found in mammalian membranes.

Tools for collection samples of fungal infections

Some of the common instruments (**tools**) used for the collection of Skin, Nail and Hair specimens for diagnosis of superficial mycotic agents. ***Scalpel, blunt end of a lancet, forceps, cello tape, and nail clipper tool.***

Wood's ultra-violet light.

Note simulated greenish-yellow fluorescence in small beaker. This device is useful as a presumptive diagnosis in the management of superficial cutaneous fungal infections.

Lab Methods Collection of Mycology specimens:

- **Skin specimens**:
  Clean with 70 % alcohol to remove dirt , oil and surface saprophytes .
- **Nails**:
  Cleaned same as skin . Usually clipped ; need to be finely minced before inoculating to media .
- **Hair**:
  Obtained from edge of infected area of scalp ; hair can be obtained by plucking , brushing , or with a sticky tape .
  A Wood's lamp can be helpful in locating infected areas . Body fluids - normal sterile collection procedures .

Scalpel *Nail Clipper Tool*

3. Nails

- Clean nail with 70% alcohol.
- Dorsal plate - Scrape outer surface and discard; scrape the deeper portion.
- Remove a portion of debris from under the nail with a scalpel.
- Collect whole nail or nail clippings.
- Place all material in a clean envelope labeled with the patient's data.

- **Body fluids:** Normal sterile collection procedures as CSF, Blood, sputum

What are the methods used to diagnose fungi? And when is it needed?

Here is the most important information.
A fungal infection is a common and not serious condition if properly diagnosed and treated early. The most prominent methods of diagnosing fungi are in the following article:
Tests used to diagnose fungi include the following:

1. Direct Microscopy Test

In this test, a sample of the affected skin or a small portion of the affected nails is scraped and examined under a microscope using one or more of the following methods:

a. Potassium Hydroxide Test.

b. Stained Dried Smear.

c. Unstained wet-mount.

d. Fluorescent staining.

e. Histopathology of biopsy.

2. Fungal Culture Test

Fungal culture analysis is used to detect superficial fungal infections that may affect the skin or nails, for example, or to detect systemic fungal infections such as those that may affect the lungs. Fungal culture analysis is done in the area where the medical provider wants to determine whether or not it has a fungal infection.

Types of fungal culture tests depending on *the site of infection* include the following:

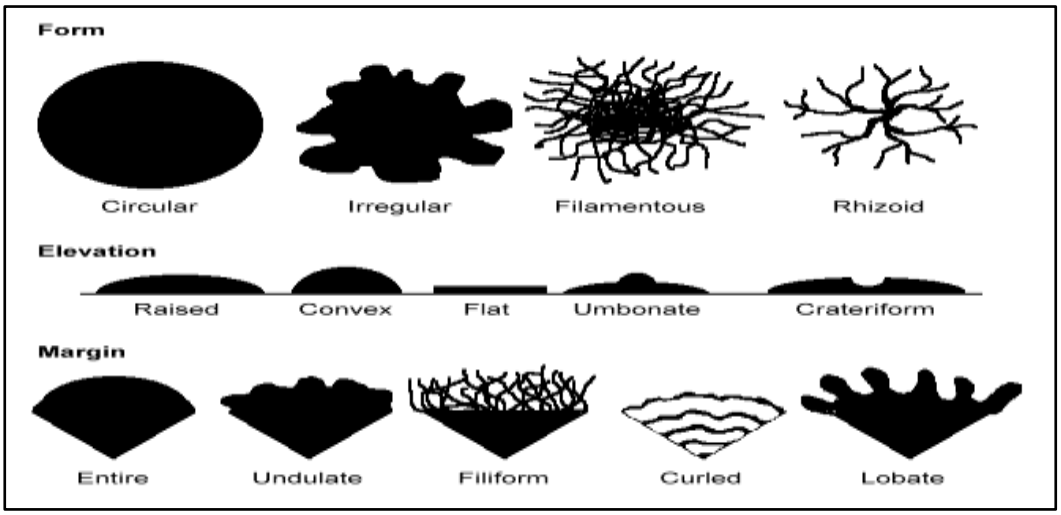
- Scraping a small part of the skin or nails to detect infection in the skin and nails.
- Taking a swab of oral or vaginal fluid to detect infection in the mouth or vagina.
- A blood test to detect more serious fungal infections such as infection in the blood.
- Sputum culture to detect fungal infection in the lungs.
- A urine test helps detect a vaginal infection.

Morphological characterization of fungal colonies structures

What are Fungal Colonies ?!!

Fungal colonies are either a mass of cells of unicellular fungi or fungal hyphae of multicellular fungi.

The main difference between bacterial and fungal colonies is that bacterial colonies are small, smooth or rough colonies with defined margins while fungal colonies are large colonies with a fuzzy appearance. Furthermore, bacterial colonies look wet and shiny while fungal colonies are powder-like.



Form : circular , Elevation : umbonate , margin: entire,

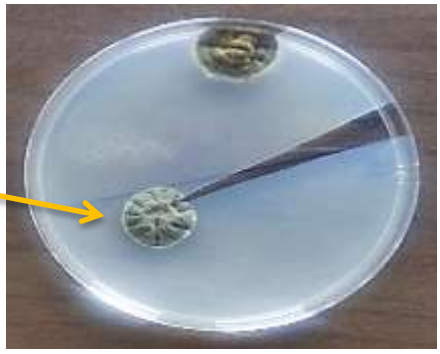
Color: black with gray.

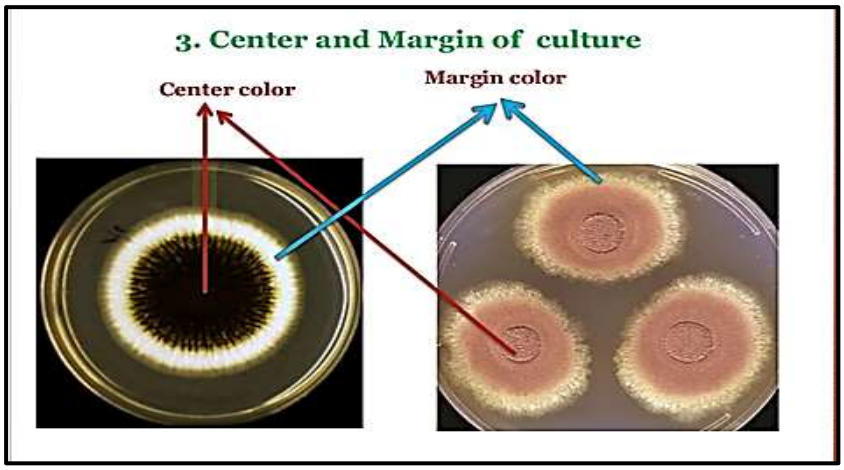
Form : irregular , Elevation : flat , margin: undulate,

Color: gray with white.

Form: circular, Elevation: raised , margin: entire,

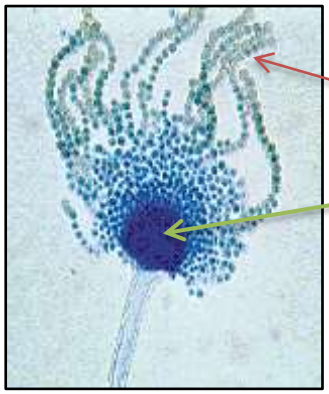
Color: green.



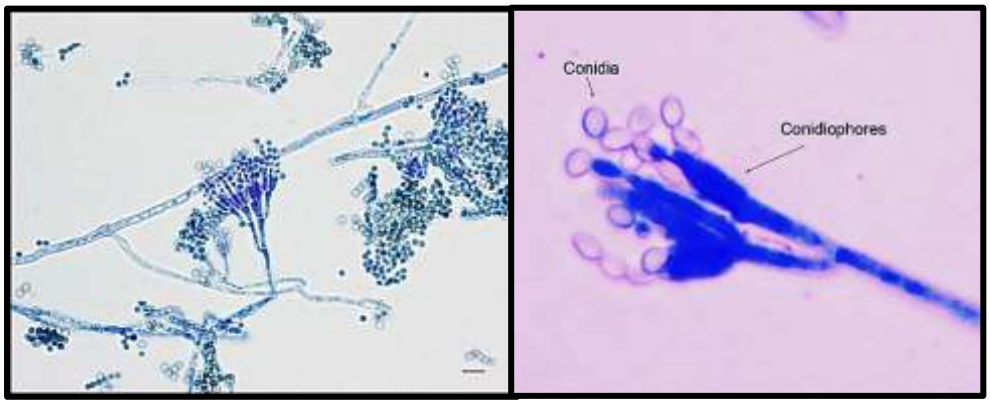


Conidiophores and conidia of Fungi: With lacto phenol cotton blue staining

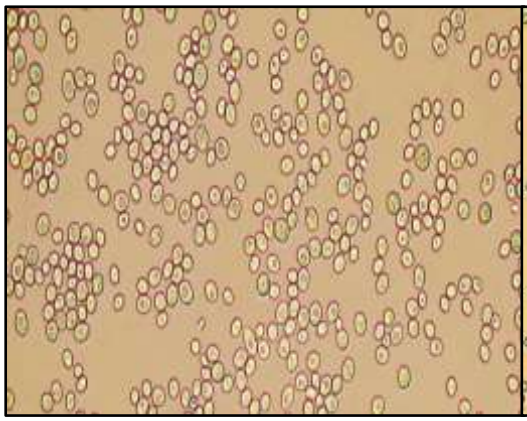
- fungi reproducing asexually by means of conidia



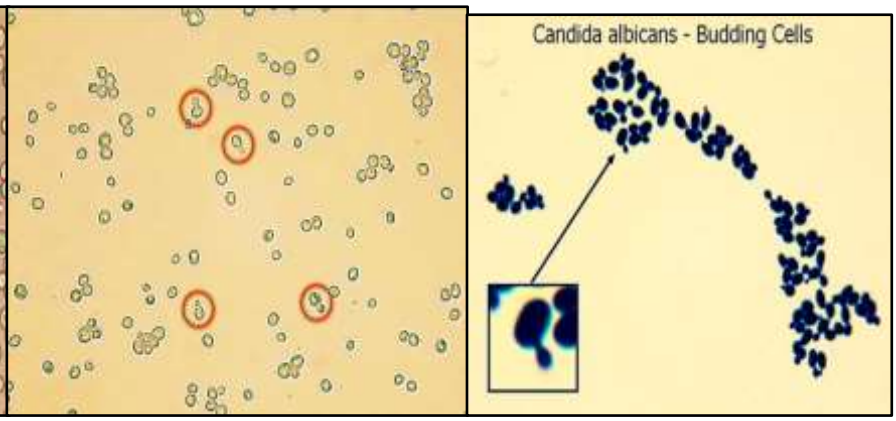
Typical appearance of the **conidial head** of *Aspergillus spp*



Penicillium spp. conidia ,under the microscope

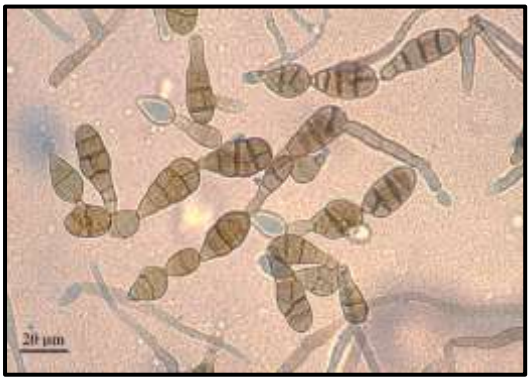


Wet mount for yeast cells with budding structure



Gram stain for yeast cells

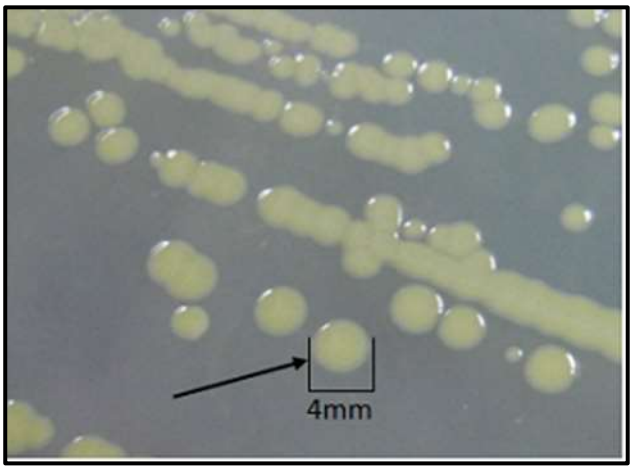
Ex. (*Candida albicans*)



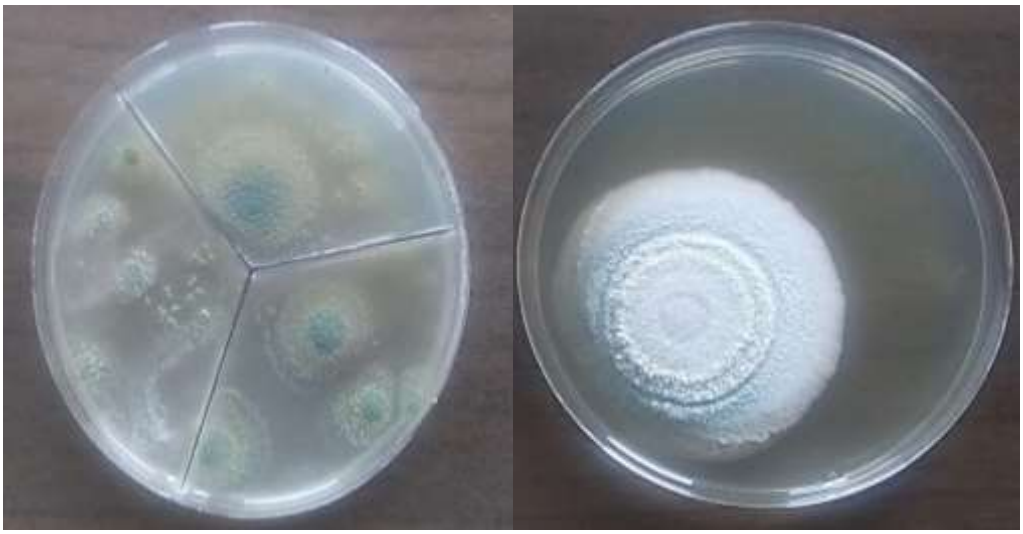
Dermatophytes spores (macroconidi)

Rhizopus spp →Rhizoid form→ ***hairy colonies***.

Candida albicans colonies (consistent white circles that aren't shiny)



Bacterial colonies are small, smooth or rough colonies with defined margins



Granules colonies ***Powdery colonies***

Ringworm infection

Antibiotic Sensitivity Test(lab.6)

▪ *What is an antibiotic sensitivity test?*

Antibiotic susceptibility testing has become a very essential step for the proper treatment of infectious diseases. Antibiotics are medicines used to fight bacterial infections. There are different types of antibiotics. Each type is only effective against certain bacteria.

Other names:

- Antibiotic Susceptibility Test .
- Sensitivity Testing.
- Antimicrobial Susceptibility Test.

Antibiotics with a wide range of influence (broad spectrum) affect multiple types of microbes, whether positive or negative for Gram. The mechanism of the anti-microbial effect varies according to their structures:
For example:

- Sulfanilamide works as similar to a substance that enters into the structure of the cell.
- Streptomycin inhibits protein with microbe.

Antibiotic resistance happens when standard antibiotics become less effective or ineffective against certain bacteria. Antibiotic resistance can turn once easily treatable diseases into serious, even life-threatening illnesses. Isolated strains that resistance antibiotics are increasing and controlling the multiplication of resistant microbe by the genes carried on plasmids, which are transmitted from one strain to another by resistance transfer factors.

▪ *What is it used for?*

1. An antibiotic sensitivity test is used to help find the best treatment for a bacterial infection(i.e. help find out which antibiotic will be most effective in treating your infection). Means to guide the clinician in selecting the best antimicrobial agent.
2. It may also be used to find out which treatment will work best on certain fungal infections, in this case is called ***antifungal.***
3. The test can also be helpful in finding a treatment for antibiotic-resistant infections. Means to accumulate epidemiological information on the resistance microorganisms of public health importance.

- ***Why do I need an antibiotic sensitivity test?***

This test is needed if you have:

1. An infection that has been shown to have antibiotic resistance or is otherwise hard to treat. These include tuberculosis, MRSA (Methicillin-resistant *Staphylococcus aureus*), and C. diff. (Clostridium enterocolitis)
2. A bacterial or fungal infection that is not responding to standard treatments.

- ***What happens during an antibiotic sensitivity test?***

The test is done by taking a sample from the infected site. The most common types of tests are listed below.

- ***Blood culture***

→ A health care professional will take a blood sample from a vein in your arm, using a small needle. After the needle is inserted, a small amount of blood will be collected into a test tube or vial.

- ***Urine culture***

→ You will provide a sterile sample of urine in a cup, as instructed by your health care provider.

- ***Wound culture***

→ Your health care provider will use a special swab to collect a sample from the site of your wound.

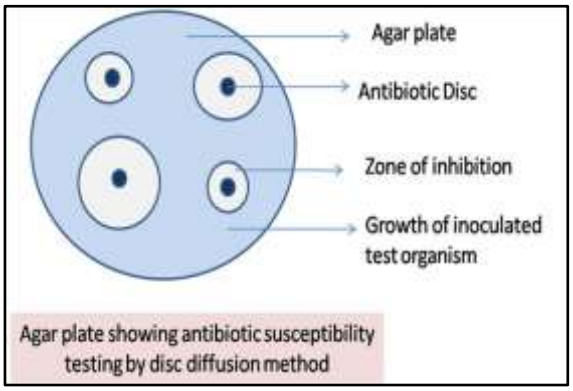
- ***Sputum culture***

→ *You may be asked to cough up sputum into a special cup, or a special swab may be used to take a sample from your nose.*

- ***Throat culture***

→ Your health care provider will insert a special swab into your mouth to take a sample from the back of the throat and tonsils.

❖ Antimicrobial susceptibility testing methods:



Agar plate showing antibiotic susceptibility testing by disc diffusion method

Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth in vitro. This ability may be estimated by either the dilution method or the diffusion method.

There are three methods :

I. Disk Diffusion. The common method used in diagnostic microbiology laboratories is modified Kirby-Bauer method.
II. Broth Dilution.
III. Agar Dilution.

Modified Kirby-Bauer Method

The method of disk sensitivity to know which type of antibiotic has an effective effect against a specific microbe. It is a fast, accurate and inexpensive diagnostic method. It is used in the laboratory to estimate the sensitivity of the clinical isolates to these antibiotics.

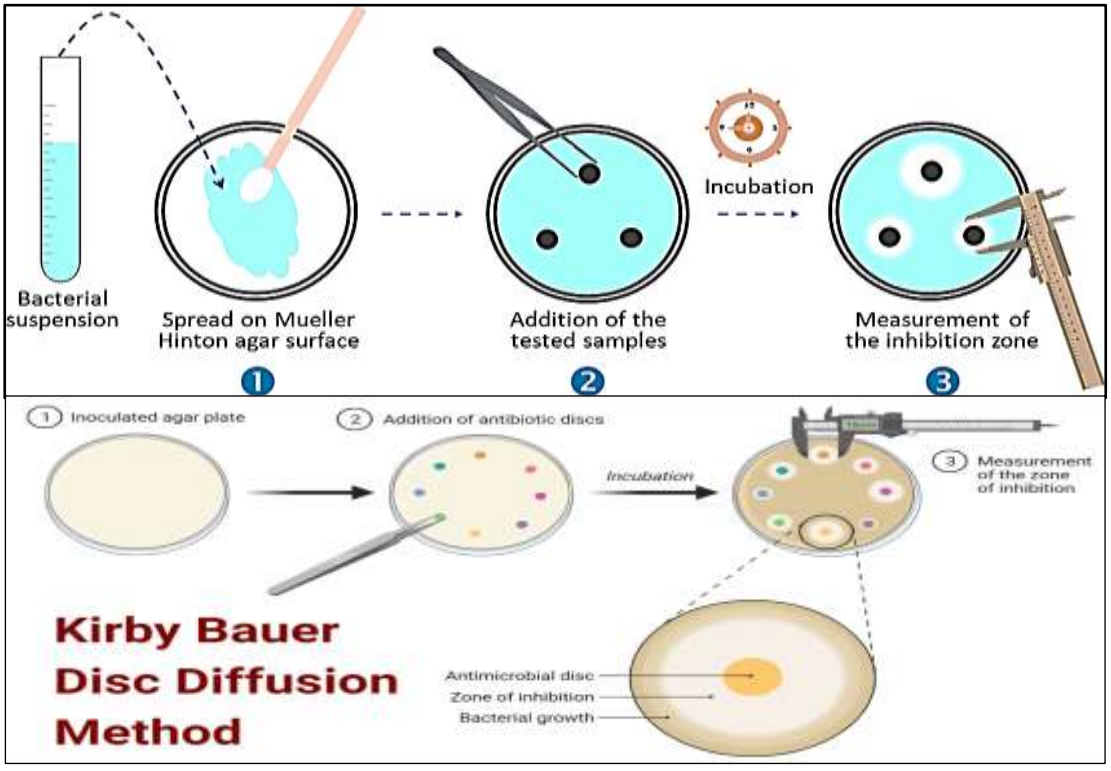
Requirements:

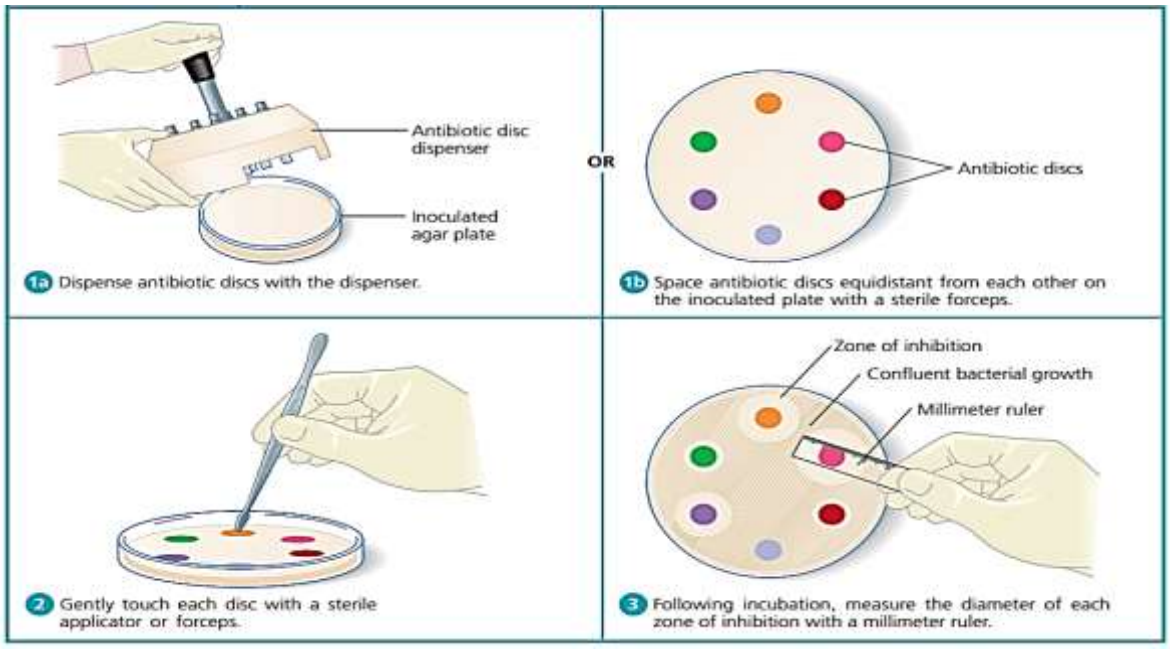
Mueller-Hinton Agar, Antibiotic Discs, Turbidity Standard (barium chloride& sulphuric acid), Swabs.

To test a specific microbe response to antibiotics.

How to work in briefly:

- The antibiotic-saturated discs are placed on the surface of the agar dish, pollinated with the microbe to be tested by sterilizer forceps or dispenser. The dish is incubated for 18-24 hours at 37 ° C.
- During incubation, the microbe grows, but growth-free areas are formed around the discs that containing antibiotics.
- The diameter of the contrast area is measured by the ruler.
- The diameter is associated with the contrast area diffusion on the agar (zone of inhibition), which is free from microbial growth, directly to the extent of the microbe response to the antibiotic affected.
- The Zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The zone of inhibition can be used to measure the susceptibility of the bacteria towards the antibiotic.




Antibiotic disc dispenser
Inoculated agar plate
1a Dispense antibiotic discs with the dispenser.
OR
Antibiotic discs
1b Space antibiotic discs equidistant from each other on the inoculated plate with a sterile forceps.
2 Gently touch each disc with a sterile applicator or forceps.
Zone of inhibition
Confluent bacterial growth
Millimeter ruler
3 Following incubation, measure the diameter of each zone of inhibition with a millimeter ruler.

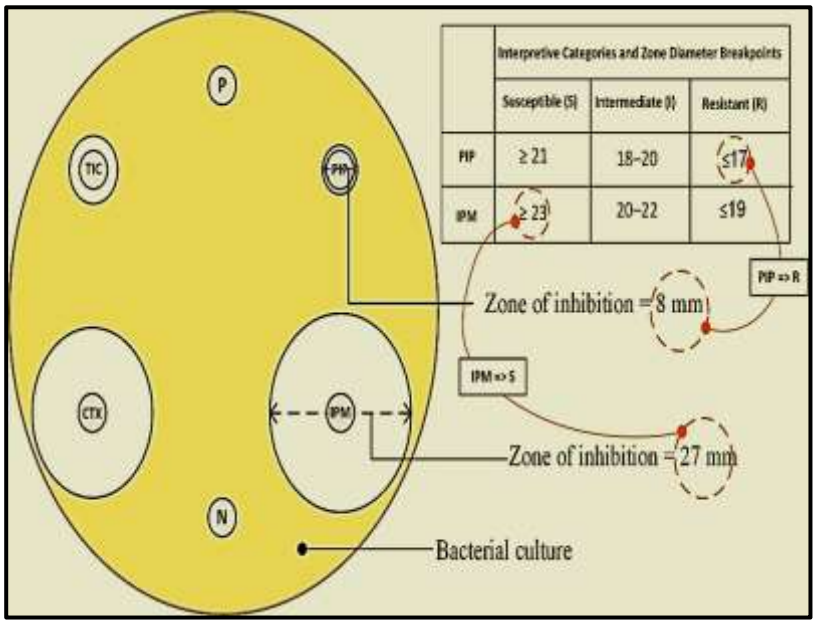
The factors that effect of inhibition zone:

Inoculated size, incubation temperature, incubation period, medium installation, pH, around air gases ex:CO_2, and antibiotic constant.

- ***What do the results mean?***

Results are usually described in one of the following ways:

- ***Susceptible(sensitive).*** The tested medicine stopped the growth or killed the bacteria or fungus causing your infection. The medicine may be a good choice for treatment.
- ***Intermediate.*** The medicine may work at a higher dose.
- ***Resistant.*** The medicine did not stop the growth or kill the bacteria or fungus causing the infection. It would not be a good choice for treatment.

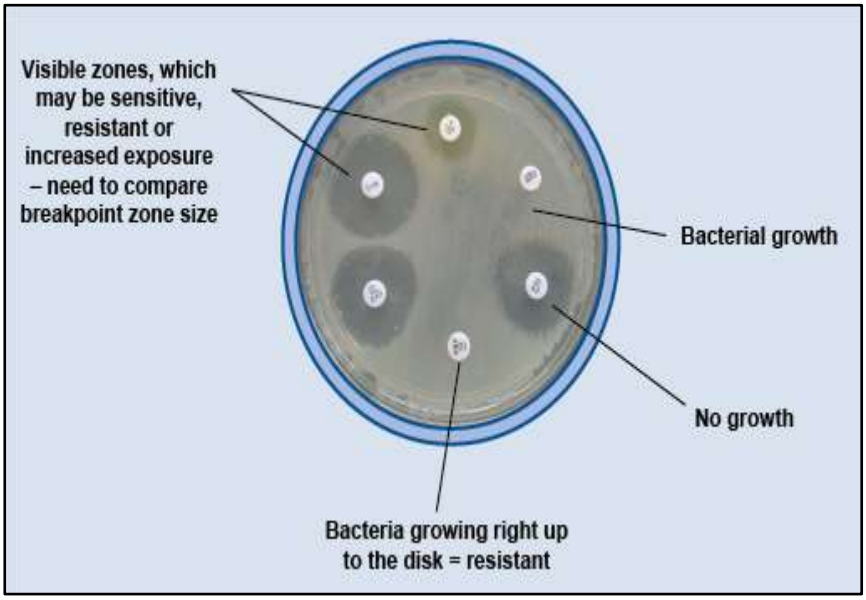


If you have questions about your results, talk to your health care provider.

- ***Is there anything else I need to know about an antibiotic sensitivity test?***

Incorrect use of antibiotics has played a big role in the rise in antibiotic resistance. *Make sure you use antibiotics the right way by:*

- Taking all doses as prescribed by your provider.
- Only taking antibiotics for bacterial infections. They don't work on viruses, like colds and flu.


Visible zones, which may be sensitive, resistant or increased exposure – need to compare breakpoint zone size
Bacterial growth
No growth
Bacteria growing right up to the disk = resistant

Holder

Calipers

Dispenser

Antibiotic discs

How to Understand Your Lab Results

1. *What is a laboratory test?*

A laboratory (lab) test is a procedure in which a health care provider takes a sample of your blood, urine, other bodily fluid, or body tissue to get information about your health.

Some lab tests are used to help *diagnose, screen, or monitor a specific disease or condition.* Other tests *provide more general information about your organs and body systems.*

Lab tests play an important role in your health care. But they don't provide a complete picture of your health. Your provider will likely include a physical exam, health history, and other tests and procedures to help guide diagnosis and treatment decisions

2. *Why do I need a lab test?*

Lab tests are used in many different ways. Your health care provider may order one or more lab tests to:

- **Diagnose or rule out** a specific disease or condition

  → An **HPV (Human papillomavirus) test** is an example of this type of test. It can show you whether or not you have an HPV infection

- **Screen for a disease.** A screening test can show if you are at a higher risk for getting a specific disease. It can also find out if you have a disease, even if you have no symptoms.

  → A **Pap test** is a type of screening test for cervical cancer

- **Monitor a disease and/or treatment.** If you've already been diagnosed with a disease, lab tests can show if your condition is getting better or worse. It can also show if your treatment is working.

  → A **blood glucose test** is a type of test that is used to monitor diabetes and diabetes treatment. It is also sometimes used to diagnose the disease.

- **Check your overall health.** Lab tests are often included in a routine checkup. Your provider may order tests of various organs and systems to see if there have been changes in your health over time. Testing can help find health problems before symptoms appear.

  → A complete blood count is a type of routine test that measures different substances in your blood. It can give your health care provider important information about your overall health and risk for certain diseases.

3. *What do my results mean?*

Lab results are often shown as a set of numbers known as a **reference range**. A reference range may also be called "normal values." You may see something like this on your results: "normal: 77-99

mg/dL" (milligrams per deciliter). Reference ranges are based on the normal test results of a large group of healthy people. The range helps show what a typical normal result looks like.

But not everyone is typical. Sometimes, healthy people get results outside the reference range, while people with health problems can have results in the normal range. If your results fall outside the reference range, or if you have symptoms despite a normal result, you will likely need more testing.

Your lab results may also include one of these terms:

- **Negative or normal,** which means the disease or substance being tested was not found
- **Positive or abnormal,** which means the disease or substance was found
- **Inconclusive or uncertain,** which means there wasn't enough information in the results to diagnose or rule out a disease. If you get an inconclusive result, you will probably get more tests.

Tests that measure various organs and systems often give results as reference ranges, while tests that diagnose or rule out diseases often use the terms listed above.

4. What are false positive and false negative results?

✓ A false positive result means your test shows you have a disease or condition, but you don't actually have it.
✓ A false negative result means your test shows you don't have a disease or condition, but you actually do.

These incorrect results don't happen often, but they are more likely to happen with certain of types tests, or if testing was not done right. Even though false negatives and positives are uncommon, your provider may need to do multiple tests to make sure your diagnosis is correct.

5. What factors can affect my results?

There are many factors that can affect the accuracy of your test results. These include:

- Certain foods and drinks
- Medicines
- Stress
- Vigorous exercise
- Variations in lab procedures
- Having an illness

If you have any questions about your lab tests or what your results mean, talk to your health care provider.